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Multicenter Clinical Evaluation of the Xpert GBS LB Assay for Detection of Group B *Streptococcus* in Prenatal Screening Specimens

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Neonatal infection with *Streptococcus agalactiae* (group B *Streptococcus* [GBS]) is a leading cause of sepsis and meningitis in newborns. Recent guidelines have recommended universal screening of all pregnant women to identify those colonized with GBS and administration of peripartum prophylaxis to those identified as carriers to reduce the risk of early-onset GBS disease in neonates. Enriched culture methods are the current standard for prenatal GBS screening; however, the implementation of more sensitive molecular diagnostic tests may be able to further reduce the risk of early-onset GBS infection. We report a clinical evaluation of the Xpert GBS LB assay, a molecular diagnostic test for the identification of GBS from broth-enriched vaginal/rectal specimens obtained during routine prenatal screening. A total of 826 specimens were collected from women undergoing prenatal screening (35 to 37 weeks' gestation) and tested at one of three clinical centers. Each swab specimen was tested directly prior to enrichment using the Xpert GBS assay. Following 18 to 24 h of broth enrichment, each specimen was tested using the Xpert GBS LB assay and the FDA-cleared Smart GBS assay as a molecular diagnostic comparator. Results obtained using all three molecular tests were compared to those for broth-enriched culture as the gold standard. The sensitivity and specificity of the Xpert GBS LB assay were 99.0% and 92.4%, respectively, compared to those for the gold standard culture. The Smart GBS molecular test demonstrated sensitivity and specificity of 96.8% and 95.5%, respectively. The sensitivities of the two broth-enriched molecular methods were superior to those for direct testing of specimens using the Xpert GBS assay, which demonstrated sensitivity and specificity of 85.7% and 96.2%, respectively.

Streptococcus agalactiae (group B *Streptococcus* [GBS]) is a Gram-positive bacterium associated with transient colonization of mucosal membranes throughout the body, including the vagina, gastrointestinal tract, and urethra (1). GBS rarely causes disease in healthy individuals but can cause serious illness in immunocompromised patients, elderly individuals, and newborn infants (2). Of particular concern is neonatal infection caused by vertical transmission during labor and birthing. Transmission from an asymptomatically colonized mother to the neonate can result in early-onset invasive GBS disease, which is a leading cause of sepsis and meningitis in newborns in the United States (3). Early-onset GBS disease in newborns can result in death or long-term disabilities such as mental retardation and hearing or vision loss (4). Because these infections are acquired through direct contact of the neonate with GBS in the mother's urogenital tract during delivery, it has become a universal practice to screen pregnant women for vaginal/rectal colonization with GBS at 35 to 37 weeks' gestation (5). The identification of GBS during routine screening results in administration of intrapartum prophylaxis to mitigate transmission of bacteria and reduce the chance of invasive disease in the newborn if GBS is transmitted vertically during the birthing process. The implementation of this screening and prophylaxis strategy has been very successful, reducing the incidence of early-onset GBS by 60 to 86% (6).

Routine culture-based screening methods rely on the collection of a vaginal/rectal swab specimen, which is then enriched using a selective broth medium such as LIM broth to achieve optimal sensitivity. Following enrichment, the specimen is subcultured onto blood agar plates (BAP) where presumptive GBS colonies are identified visually by characteristic zones of narrow beta-hemolysis. The identification of the presumptive colonies is confirmed using phenotypic and biochemical methods (5). A limitation to this method is that approximately 5 to 8% percent of all GBS isolates do not produce β-hemolysin (7–9). The lack of characteristic hemolysis in these strains can lead to a false-negative culture result (9). Alternative screening media, including chromogenic Granada agar, have been validated for identification of GBS. The GBS colonies grown on Granada agar produce an orange carotenoid pigment (granadaene), which allows easy identification of GBS colonies. While this test is more sensitive than subculture to blood agar, most non-hemolytic GBS strains also fail to produce granadaene (9). A failure to identify granadaene-negative and nonhemolytic strains may result in up to 5% of colonized women not receiving appropriate prophylaxis. Finally, culture-based screening methods require broth enrichment for optimal sensitivity. This extends the turnaround time (TAT) for results to 48 to 72 h. Because screening cultures are collected at 35 to 37 weeks' gestation, this delay does not typically impact patient man-

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agement. However, up to 15% of pregnant women do not receive adequate prenatal care including routine screening for GBS (10, 11). In these cases, a more rapid and sensitive direct testing method for screening may be desirable.

We conducted a multicenter evaluation of the FDA-cleared Xpert GBS LB assay (Cepheid, Sunnyvale, CA) for detection of GBS from broth-enriched vaginal/rectal swabs. This test utilizes real-time PCR to detect a conserved target within the 3′ DNA region adjacent to *cfb*, the gene encoding the *S. agalactiae* CAMP factor (12–14). Results were compared to LIM broth-enriched culture with plating on 5% sheep blood agar as a gold standard. The sensitivity and specificity of the Xpert GBS LB assay were also compared to those of a second molecular test, the Smart GBS (Cepheid), conducted on broth-enriched specimens, and to those of direct analysis of nonenriched swab specimens using the Xpert GBS assay (Cepheid).

MATERIALS AND METHODS

Collection of vaginal and/or rectal specimens. Two paired vaginal/rectal swabs were collected from patients at 35 to 37 weeks of gestation undergoing routine GBS screening. A total of 861 specimens were collected and tested at 3 separate trial sites (The Medical College of Wisconsin, Indiana University School of Medicine, and Sacred Heart Hospital) in accordance with site-specific institutional review board (IRB)-approved protocols. Only one set of swabs per patient was tested to avoid duplicate results. One of the two swabs was removed from the transport medium and used to test direct specimens on the Xpert GBS assay prior to LIM broth enrichment. The second swab was used for inoculation of LIM broth for specimen enrichment. After 24 h of enrichment, the LIM broth was used to test in parallel the Xpert GBS LB and Smart GBS assays and reference cultures. The collection and use of clinical specimens for this study were approved by the IRB at each participating clinical center.

Xpert GBS assay and Xpert GBS LB assay. One of two paired rectal/vaginal swab specimens was transferred to the designated chamber of the Xpert GBS assay cartridge. The swab was snapped at the score mark, and the cartridge was loaded into a Cepheid GeneXpert system (GeneXpert IV, GeneXpert XVI, Infinity 48, or Infinity 80 system) for automated sample preparation and PCR. The results for specimens are reported as positive or negative based on the detection of a genomic target sequence adjacent to the *S. agalactiae cfb* gene. The total assay run time was 55 min with <1 min of hands-on time. Specimens with tests that yielded invalid results were retested once by transferring the remaining fluid in the loading chamber to a new cartridge. If no fluid remained in the invalid cartridge, the swab was transferred to a new cartridge, using sterile tweezers, and a single repeat test was performed. The second paired swab was used to inoculate LIM broth for culture enrichment. A sterile swab (provided by Cepheid) was dipped into the broth-enriched culture and then transferred to a designated chamber and was processed the same as the direct method. The positive and negative controls were run on each day of testing for the clinical study; however, once verified by a clinical laboratory, controls may be reduced in frequency according to the College of American Pathologists (CAP) guidelines for single-use assays with internal controls (once every 30 days or once every new lot of cartridges is tested).

Smart GBS assay. A 200-μl aliquot of each LIM broth-enriched specimen was transferred to a lysis tube (provided), mixed, and centrifuged at 10,000 × *g* for 3 min. The supernatant was removed, and 750 μl of diluent reagent (provided) was added to resuspend the cell pellet. Samples were vortexed for 5 min to liberate nucleic acid, and a 5-μl portion of the lysate was added to a master mix tube along with 21 μl of diluent to reconstitute lyophilized PCR reagents. A 25-μl portion of the reaction components (mixed master plus specimen) was transferred to a SmartTube and tested using the SmartCycler DX system, following the recommended thermocycling parameters for the Smart GBS assay. A positive control and a negative control were included with each Smart GBS run. In addition, the Smart GBS assay includes an internal control to confirm the proper amplification in each real-time PCR. Specimens are reported as positive or negative based on the parameters set in the SmartCycler protocol. Invalid results were repeated once using residual specimen lysates.

Broth-enriched cultures for identification of GBS. As a gold standard in this study, one of two paired swabs was inoculated into LIM broth for 16 to 24 h at 35 to 37°C. The broth-enriched cultures were subcultured to BAP and incubated at 35 to 37°C for up to 48 h. The broth-enriched subcultures were examined for the presence of beta-hemolytic colonies as a presumptive identification of GBS. The identification was confirmed using a Gram stain (Gram-positive cocci in chains), catalase (nonreactive), and a group B *Streptococcus* latex agglutination test (Streptex; Remel, Lenexa, KS).

Discrepant result analysis. A discrepant result was defined as a result obtained with the Xpert GBS, Xpert GBS LB, or Smart GBS assay that did not correlate with the culture results. The molecular test-positive, culture-negative or molecular test-negative, culture-positive discrepant results were resolved using bidirectional sequence analysis of the GBS *cfp* target with primers different from those used for the Xpert GBS and Smart GBS assays.

Statistical analysis. The results from the Xpert GBS LB and Smart GBS assay were compared to culture results as a gold standard. The performance characteristics, including sensitivity and specificity, were calculated using standard methods. The 95% confidence intervals (CI) were calculated by using a binomial expansion.

RESULTS

Comparison of Xpert GBS LB assay to enriched culture. A total of 861 subjects were enrolled at three clinical centers to test the ability of the Xpert GBS LB assay to detect GBS from broth-enriched cultures. Thirty-five specimens were excluded from the analysis due to failure in meeting the predetermined inclusion criteria. These included 11 (1.2% of total samples tested) specimens with LIM broth inoculated >24 h after swab collection, 11 (1.2%) specimens with LIM broth incubated >24 h, 8 (0.9%) specimens without broth enrichment culture, 2 (0.2%) specimens tested using incorrect or expired Xpert test cartridges, 2 (0.2%) mislabeled cultures, and 1 (0.1%) specimen without a reference culture result. This reduced the total number of protocol-compliant specimens to 826, which were used to generate performance data. Compared to the reference method, the sensitivities of the Xpert GBS LB assay at the three clinical sites were 98.6% (73/74), 97.8% (44/45), and 100% (72/72) (Table 1). Combined, these data resulted in 99.0% overall sensitivity (189/191) with a 95% CI of 95.9% to 99.8%. Sequence analysis of the two false-negative results failed to identify GBS.

A total of 48 culture-negative, Xpert GBS LB assay-positive specimens were identified at the three clinical sites, resulting in an overall specificity of 92.4% (587/635). Two clinical centers found similar specificities of 94.1% and 95.4%, while the third center reported a lower specificity of 88.2%. Of the 48 false-positive results, 47 (97.9%) were available for discrepant analysis using nucleic acid sequencing. The sequence analysis confirmed the presence of GBS in 89.4% (42/47) of the discrepant specimens analyzed. Further, 24/47 were also positive when tested using the Smart GBS assay. These findings support the presence of GBS nucleic acid within these specimens and raise the combined specificity of Xpert GBS LB assay to 99.2% (629/634) for evaluable specimens. The remaining specimen was unavailable for discrepant analysis.

Direct testing of swab specimens using the Xpert GBS assay. Two of the three clinical sites compared the ability to detect GBS

TABLE 1 Performance of the Xpert GBS LB assay for analysis of LIM broth-enriched specimens compared to broth-enriched culture

Clinical test site	No. of specimens at site	No. of specimens with result of:[a]				Sensitivity (% [CI])[b]	Specificity (% [CI])
		TP	TN	FP	FN		
A	295	73	195	26	1	98.6 (92–99)	88.2 (83–92)
B	220	44	167	8	1	97.8 (87–100)	95.4 (91–98)
C	311	72	225	14	0	100 (934–100)	94.1 (90–97)
Total	826	189	587	48[c]	2[d]	99.0 (96–100)	92.4 (90–94)

[a] TP, true positive; TN, true negative; FP, false positive; FN, false negative.
[b] CI, 95% confidence interval.
[c] Forty-two of 47 specimens were confirmed as positive for GBS and 5/47 as negative for GBS following discrepant analysis by nucleic acid sequencing. Additionally, 24/47 specimens were positive when tested with the Smart GBS assay. One specimen was not available for discrepant analysis.
[d] Both specimens were confirmed as negative for GBS following discrepant analysis by nucleic acid sequencing.

directly from swab specimens to detection following broth enrichment using the Xpert GBS and Xpert GBS LB assays, respectively. A total of 505 enrolled subjects were available for direct testing, and the results were compared to those for the reference culture. The sensitivity of direct testing was 85.7% (96/112) overall, with sensitivities of 82.9% (34/41) and 87.3% (62/71) reported at individual sites (Table 2). The specificity was determined to be 96.2% (378/393) overall, with 98.1% (153/156) for one site and 94.9% (225/237) for the second site. This is in comparison to a combined 99.1% (116/117) sensitivity for broth-enriched cultures tested at these two sites. These data indicate a statistically significant ($P < 0.001$) decrease in sensitivity for testing of direct (swab) specimens compared to that for testing conducted after broth enrichment.

Comparison of Xpert GBS LB assay to Smart GBS assay for detection of GBS from broth-enriched culture. Broth-enriched swab specimens tested using the Xpert GBS LB assay were also tested using another FDA-cleared molecular test, the Smart GBS assay, for detection of GBS. Twelve specimens could not be tested in accordance with the Smart GBS package insert and were excluded from analysis. Compared to reference culture methods, the sensitivity and specificity of the Smart GBS assay were 96.8% (184/190) and 95.5% (595/623), respectively (Table 3).

Three of the six specimens categorized as false negative based on the Smart GBS result were available for sequence analysis. Two of these specimens were found to be positive for the presence of GBS nucleic acid, while the other one was negative. The remaining 3 specimens were not available for discrepant analysis. For the sequence analysis of 26 specimens categorized as resolved, 24/26 were positive for GBS and 2/26 were negative. Two specimens categorized as false positive were not available for discrepant analysis. These data support the presence or absence of GBS nucleic acid in these specimens and raise the combined sensitivity and specificity to 99.0% and 99.6%, respectively, for the Smart GBS assay.

Xpert GBS LB assay analytical success rate. The Xpert GBS LB assay had an initial test success rate of 98.1% (810/826). The indeterminate cases included 12 error results, 2 invalid results, and 2 no result outcomes. All indeterminate specimens were retested and yielded valid results upon a single retest, raising the final success rate to 100%. The direct testing of swabs resulted in an initial test success rate of 90.6% (471/520). A single retest of the initially unresolved specimens resulted in 34/48 valid results, giving a final call rate for direct testing of 97.1% (505/520). The test for one specimen with an initial invalid result could not be repeated. Of note, there was a significant difference in the direct testing success rates between the two sites. Site B had an initial test success rate of 78.3% (166/212), while site C had an initial test success rate of 99.0% (305/308). Site B received specimens from 14 clinic sites. The error/invalid rate for specimens received from these sites varied from 0% to 57%, suggesting that variance in specimen collection practices by specific providers or clinics (such as the use/amount of lubricant or other material used during collection of the specimens) may impact the assay call rate. Unfortunately, because of the deidentified nature of the specimens, we were not able to further investigate this hypothesis. Alternatively, the low initial success rate observed at site B may be related to the training and familiarity of the staff with the assay. Site C routinely uses the Xpert GBS assay for the direct analysis of swab specimens as a standard of care while site B does not routinely use this assay. Other publications have noted that the rates of invalid results decrease over time as sites gain experience with the assay (15).

DISCUSSION

The implementation of universal screening of pregnant women at 35 to 37 weeks' gestation has significantly reduced the risk of early-onset GBS infection in neonates from 1.5 per 1,000 births in the 1980s to 0.34 per 1,000 births in recent years (4, 5). Several studies have been performed to identify the cause of the remaining infections. A major contributing factor is the failure to seek prenatal care, including routine GBS screening, which affects up to 15% of

TABLE 2 Performance of the Xpert GBS assay for analysis of direct swab specimens compared to broth-enriched culture

Clinical test site	No. of specimens at site	No. of specimens with result of:				Sensitivity (% [CI])	Specificity (% [CI])
		TP	TN	FP	FN		
B	197	34	153	3	7	82.9 (67–92)	98.0 (94–100)
C	308	62	225	12	9	87.3 (77–94)	94.9 (91–97)
Total	505	96	378	15	16	85.7 (78–91)	96.2 (94–98)

TABLE 3 Comparison of the Smart GBS and Xpert GBS LB assays to the broth-enriched culture

Test	No. of specimens	No. of specimens with result of:				Sensitivity (% [CI])	Specificity (% [CI])
		TP	TN	FP	FN		
Smart GBS	813	184	595	28[a]	6[b]	96.8 (93–99)	95.5 (94–97)
Xpert GBS LB	826	189	587	48[c]	2[d]	99.0 (96–100)	92.4 (90–94)

[a] Twenty-four of 26 specimens were confirmed as positive for GBS and 2/26 as negative for GBS following discrepant analysis by nucleic acid sequencing. Two specimens were not available for discrepant analysis.
[b] Two of 3 specimens were confirmed as positive for GBS and 1/3 as negative following discrepant analysis by nucleic acid sequencing. The remaining 3 were not available for discrepant analysis.
[c] Forty-two of 47 specimens were confirmed as positive for GBS and 5/47 as negative following discrepant analysis by nucleic acid sequencing. Additionally, 24/47 were positive when tested with the Smart GBS assay. One specimen was not available for discrepant analysis.
[d] Both specimens were confirmed as negative for GBS following discrepant analysis by nucleic acid sequencing.

all pregnant women (16). Another complicating factor is that women may be colonized in the days or weeks between the time of the routine GBS screening and delivery. In these cases, the neonate is at risk of infection despite the negative screen result (17, 18). One study has shown that >70% of early-onset neonatal GBS infections are associated with mothers whose colonization status was either unknown or negative at the time of screening (35 to 37 weeks' gestation) (17). In these instances, use of a rapid method for the detection of GBS at the time of delivery may help determine if the administration of prophylaxis during delivery is warranted (16).

This multicenter clinical evaluation of the Xpert GBS LB assay for the identification of GBS included a total of 826 samples that were qualified for the established study criteria. The overall prevalence of GBS colonization was 23.1% (ranging from 20.4 to 25.0%) as determined by the LIM broth-enriched culture method. The Xpert GBS LB assay was more sensitive than the culture method as demonstrated by the identification of 42 specimens that were positive by the Xpert GBS assay but negative by the combined culture methods. The additional 42 GBS-positive specimens identified by the Xpert GBS LB assay may be attributable to a low concentration of GBS in the specimen which was not recovered by culture or to the identification of non-beta-hemolytic GBS that were missed by culture. Previous studies have demonstrated decreased recovery of GBS following broth enrichment of specimens containing a high concentration of *Enterococcus* spp., which overgrow GBS in broth and hinder recovery upon subculture (19, 20). Alternatively, these additional positive results might correspond to a nonviable organism or a residual GBS nucleic acid in the specimen from previous carriage. It is unlikely that these results were due to template or amplicon contamination of the Xpert LB test since 42/47 (89.4%) of specimens resolved using nucleic acid sequencing were found to be positive for GBS. The Xpert GBS LB assay also demonstrated increased sensitivity compared to that of the Smart GBS assay (99.0% [CI, 96.3% to 99.9%] and 96.8% [CI, 93.3% to 98.8%], respectively (Table 3); however, this difference was not statistically significant ($P = 0.15$). The specificity of the Xpert GBS LB assay was lower than that of the Smart GBS assay (92.4% compared to 95.5%); however, this difference was also not statistically significant.

Direct testing from the collection swab demonstrated a reduced sensitivity of 85.7% compared to those of the two broth-enriched molecular methods. Other studies that have compared the Xpert GBS assay to culture have found sensitivities ranging from 91.0% to 99.6% (21, 22). In one study, a selective medium was used, and in another, any nonhemolytic colonies that had similar colony morphology of GBS were further characterized. These differences might explain the difference in sensitivities between studies because the comparison assays were more sensitive than BAP alone. Although results are significantly less sensitive than those for enriched specimens, the ability to test a direct specimen may aid in guiding administration of prophylaxis in the 10 to 15% of patients who have not been screened prior to delivery, in cases of unexpected or early labor where enrichment culture results are not yet available, or as a method for identifying women who were colonized between routine screening and time of delivery. A number of studies suggest, through indirect observation, that low-level carriage of GBS is associated with a reduced transmission rate compared to that in patients with higher bacterial burdens (23–25). In these cases, a rapid, direct molecular test would likely identify those women most at risk of transmission of GBS to the neonate despite the reduced sensitivity compared to that of testing of enriched cultures. Challenges other than the limits of detection (LOD) can also affect the ability to test primary swab specimens using PCR. These include the presence of mucus or lubricant in a specimen, variable specimen viscosities, or the presence of blood or other materials that can physically interfere with the microfluidics of an assay such as the Xpert GBS or directly inhibit PCRs. The current protocol for direct swab testing does not involve any processing of the sample that might help in overcoming these barriers such as the dilution or filtration of specimens. This, combined with user variability, may explain the high indeterminate result rate observed at one test site during direct testing with the Xpert GBS assay.

A strength of this study is the large number of tested samples ($n = 826$) along with the collection and testing of specimens at three separate sites. The statistically equivalent performance of the Xpert GBS LB assay at three separate sites (sensitivity of 97.8% to 100% and specificity of 88.2% to 95.4%) demonstrates that the assay is a reliable diagnostic device, independent of external variables such as laboratory personnel, laboratory workflow, and specimen collection practices. An advantage of the Xpert GBS LB and Xpert GBS assays is the sample-to-result capability. Compared to the Smart GBS assay, this moderate complexity setup of the assay allows for minimal hands-on time, requiring <2 min per sample. In addition, specimen lysis, nucleic acid extraction, target amplification, and detection are performed within the test cartridge. This automation reduces the chance of contamination during handling and reduces the time to the result compared to those of other molecular tests that rely on manual specimen processing and setup of multiple real-time PCRs.

A commonly cited drawback of incorporation of molecular

tests for routine GBS screening is the higher cost than that of bacterial culture methods (26). Because screening for GBS colonization generally takes place 3 to 5 weeks prior to delivery under the current guidelines, rapid detection is not routinely necessary and may not justify the increased cost for molecular detection. A study by Berg et al. compared five different approaches for detecting GBS from vaginal/rectal swabs at 35 to 37 weeks' gestation, including Northeast Laboratory GBS agar (Northeast Laboratory Services, Winslow, ME), GBS Detect (Hardy Diagnostics, Santa Maria, CA), and GBS PCR from enriched LIM and carrot broth cultures (27). The researchers demonstrated that enriched PCR methods could yield quicker results, but there was no significant improvement in GBS detection. In addition, the cost of reagents and labor was 13 times more for PCR detection than for the use of chromogenic media. The use of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for identification of bacterial and fungal isolates has been shown to cost less than $1 per specimen and reduces the overall cost of identification by approximately 57% (28, 29). However, this technology currently requires a pure bacterial isolate, which renders it inapplicable for identification of GBS in enrichment broth.

Although the additional cost of PCR-based identification of GBS may not be efficient for screening at 35 to 37 weeks' gestation, two recent studies have demonstrated a cost benefit of implementing molecular testing at the time of delivery. One study performed a cost-effectiveness analysis comparing the current 35 to 37 weeks' screening to PCR screening at the time of delivery (17). This study estimated that the current screening method resulted in unnecessary antibiotic prophylaxis for 13.6% of pregnant women in the study compared to 4.5% for women using the intrapartum PCR test. This resulted in incremental costs of €36 and €173 to the health care system and hospital, respectively, for each mismanaged patient. Furthermore, the overuse of antibiotics in 13.6% of all patients may lead to antibiotic-resistant strains. The current studies have not identified significant penicillin resistance, but depending on the region, 10 to 38% and 5.0 to 51% of isolated GBS have resistance to erythromycin and clindamycin, respectively (30, 31). A second study found that PCR screening during pregnancy would be cost neutral and may result in an overall reduction in early-onset GBS disease (32). A small reduction in the incidence of early-onset GBS disease based on the use of a molecular test might also justify the additional cost because, on average, the cost of an early-onset GBS infection is >€19,000, and infants who survive incur health care and social care costs twice as high as those for unaffected infants (33).

The results of this study support the increased sensitivity of molecular testing and add to the literature further evidence that the use of molecular methods for the detection of GBS colonization might increase the number of recognized carriers, which may further reduce the rate of early-onset GBS infection.

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